Measurement of GFP Expression and DNA Content in Permeabilized Cells

Reagents

- Cells to be studied expressing green fluorescent protein (GFP). Note that the same cell type without GFP is needed as a control.
- 1 X PBS
- 2% Buffered formaldehyde solution (see recipe)
- 70% Ethanol
- Propidium Iodide stock solution (1mg/ml in PBS)
- DNAse-free ribonuclease A
- 12 X 75 mm culture tubes
- Vortex mixer
- Water bath at 37^{o}C

Method

Fix cells with formaldehyde

1. Count cells.
2. Place approximately 10^{6} cells into a 12 x 15 mm test tube and wash them once with PBS by centrifugation for 5 min at 300 x g at 2-8^{o}C.
3. Remove supernatant by aspiration or rapid decanting and add 500 ml of cold PBS to the cell pellet. Mix gently. Add 500 ml of cold, buffered 2% formaldehyde solution and mix again. Incubate at 2-8^{o}C for 1h.

Permeabilize cells with ethanol

4. Spin cells down by centrifugation for 5 min at 300 x g at 2-8^{o}C, remove supernatant by aspiration or rapid decanting, wash once with cold 1 X PBS, then add 1 ml of 70% ethanol at – 20^{o}C drop-wise to the cell pellet with the tube sitting on a vortex. Incubate cell suspension overnight at 2-8^{o}C.
5. Spin cells down by centrifugation for 5 min at 300 x g at 2-8^{o}C, remove supernatant by aspiration or rapid decanting and add 1 ml of a solution containing 40µg/ml of PI and 100 µg/ml of ribonuclease A. Incubate cell suspension at 37^{o}C in the dark for 30 min. If needed, filter samples through a nylon mesh to remove clumps before acquisition on the flow cytometer.

Preparation of Buffered 2% Formaldehyde Solution

Add 2g paraformaldehyde (Polysciences, Inc.) to 100 ml PBS. Heat the solution to 70^{o}C in a fume hood until the paraformaldehyde goes into solution (approximately 1 h). Cool to room temperature, check pH and adjust to 7.2 with 0.1M NaOH or 0.1M HCl. Store at 2-8^{o}C protected from light. The solution is stable for at least 1 month. Check pH periodically. Do not heat the solution above 70^{o}C. For best results, use only very pure preparations of paraformaldehyde (i.e., electron microscopy grade from Polysciences).

References

Chu, YW, Wang R., Schmid I, Sakamoto KM. Analysis with flow cytometry of green fluorescent protein expression in leukemic cells. *Cytometry* 36:333-339, 1999.

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